Root-derived biopolymers in Legumes

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INTRODUCTION

Given the limited mobility of plants, a variety of carbon- and nitrogen-based polymers must be articulated to provide structural rigidity and defense from pathogens, while at the same time allowing the root system to proliferate when environmental conditions and nutrients are optimum. *Cellulose*, an abundant crystalline glucose-based polymer is a major source of fiber, whether in the form of cotton, or as wood-pulp for the paper industry. Several waxes, such as *cutin* and *suberin*, are heavy lipids coating the exterior of fruits, leaves, seeds and roots, preserving moisture and protecting plant tissue from fungal infections. Several large classes of plant-derived polymers are less well known, but will be essential building blocks for agricultural biotechnology of industrial feedstocks [1]. *Xylan* is the predominant non-crystalline polysaccharide of hardwoods and agricultural crops, and is a polymer of xylose. It is primarily found in the hemicellulose fraction of plant cell walls, and is responsible for the swelling properties and biodegradation characteristics of biomass.

MATERIALS AND METHODS

Mung bean (*Vigna mungo* L.) seedlings were germinated in sand directly in IR-transmissive root boxes as described previously [2,3], and fertilized weekly with complete inorganic nutrients. Infrared spectromicroscopy at Beamline 1.4.3 allowed acquisition of spectra over the wavelength region of 2.5 to 16 microns (4000 - 650 cm^{-1}) from intact root systems coincident with the ZnSe windows over time periods of 3 d to 60 d after seed germination.

RESULTS

Figure 1 shows a comparison of mid-IR spectra (shown in black) taken from a recently germinated mung bean seedling grown in sand with the library spectrum of xylan (shown in red).

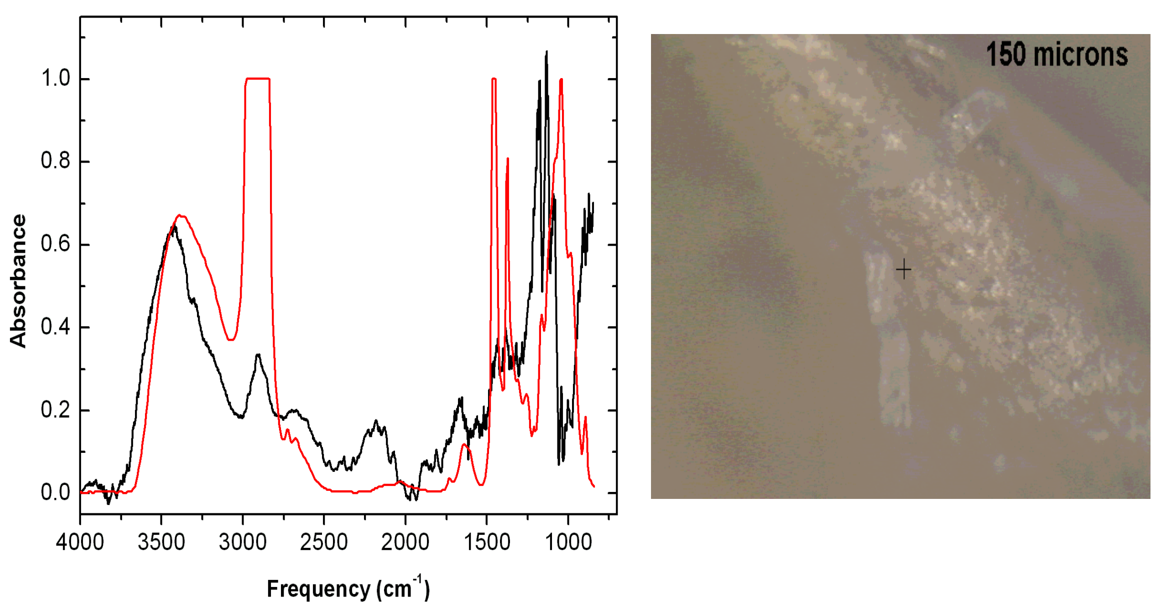

Figure 1

To the right appears a visual photomicrograph through the 15x objective of the IR microscope of the field. Additional absorbances at 1189, 1179, 1173 and 1132 cm^{-1} in the mung bean spectrum demonstrates that the seed-expressed polymer is a complex mixture of xylans and mannose/galactose-derived linkages. These polymers are extremely hydrophilic, and assist in absorbing water for seed germination.

Figure 2 is a visual image through the IR microscope with the 15x objective of the root zone of mung bean demonstrating an elaborate *mucilage* array surrounding the primary root, which is oriented horizontally in the picture. Mucilage is a complex, hydrophilic polysaccharide exuded by growing plant roots, and allows roots to penetrate compact soils, as well as providing food for microbial growth. To the right of the photomicrograph is the mid-IR spectrum from a 5 micron x 10 micron region of the mucilage network. This IR signal has spectral features arising from a complex mixture of proteins, aromatic carboxylic acids and sugars [4]. The amide-I modes of proteins have a characteristic feature at ~1650 cm^{-1}, with additional amide absorptions at 1440 and 1335 cm^{-1}. Primary alcohols possess IR features from 1030-1060 cm^{-1} (and at lower energies with unsaturation). Previous studies of legume responses to low-phosphorus document a general acidification of the rhizosphere, and increased exudation of aliphatic and aromatic carboxylic acids. The identity of the protein(s) inhabiting the soil-solution at high concentration is as yet unknown, but extracellular phosphatases have been observed in solution-culture experiments with tobacco, and the protein spectra acquired to date from the mucilage indicate glyco-linked hydrolytic enzymes. The picture that emerges from such studies is of a dynamic rhizosphere, constantly synthesizing and degrading polysaccharides. The subtler, plant-plant and plant-microbe aspects of this chemical communication pathway will be the subjects of future studies.

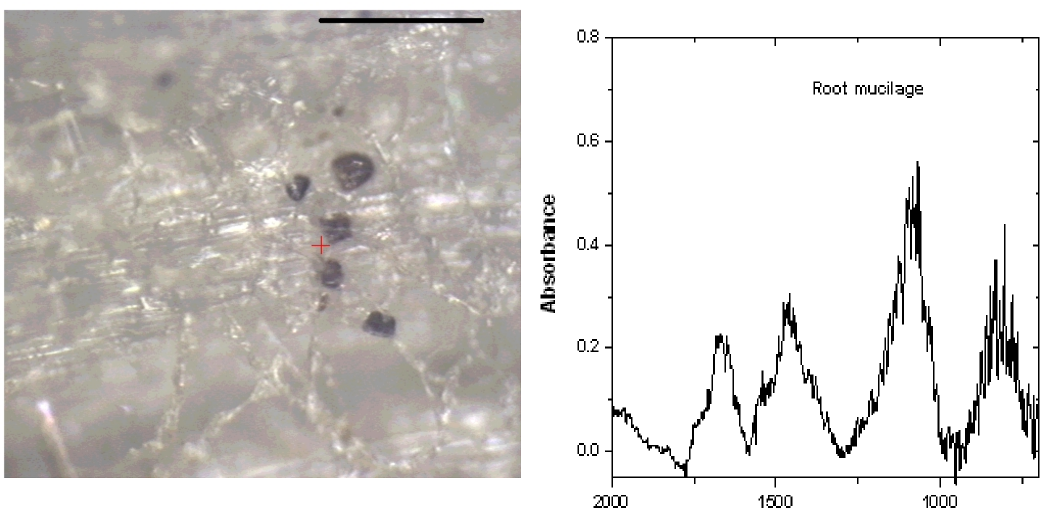

Figure 2.

ACKNOWLEDGEMENTS

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